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THE COMPARISON OF A SYNDROME OF SHEEP, RATS AND RABBITS ON SIMILAR DEFICIENT RATIONS

By

Nick Elia Nekoliczuk

A THESIS

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The COMPARISON OF A SYNDROME OF SHEEP, RATS AND RABBITS ON SIMILAR DEFICIENT RATIONS.

INTRODUCTION

The use of small laboratory animals in solving nutritional problems that have developed in the feeding of farm animals has increased. The financial saving effected in housing, feeding and labor costs has been largely responsible for the general movement in this direction. The ease with which large numbers of these animals can be housed and handled in a limited space combined with the reduced experimental error as a result of the many individuals which can be conveniently used makes this class of experimental material extremely popular. The fact that less time is required to carry out certain experimentation with laboratory animals is an obvious advantage. The short gestation period of laboratory animals allows for numerous pregnancies and lactations in a relatively much shorter time. Since these processes constitute the severest form of depletion treatment, deficiency symptoms appear within a relatively short period of time in the smaller animals. Lastly, the results of small animal experimentation serve a useful purpose in the substantiation and confirmation of data recorded in farm animal projects. After the latter has yielded definite data, the experiments may be paralleled by small animal trials, and within a relatively short time confirmatory evidence is available to add greatly to the authenticity of the results secured with larger animals.

Nutritional deficiency studies involving the use of laboratory animals may arise in one of two ways, either directly or indirectly. By the direct method groups of small animals are used experimentally to study some nutritional problem. From the results deductions are made as to what may be expected in farm animals. By the indirect method a problem is studied with some species of farm animal to a point where certain definite observations are made that require further study. To continue the study of the particular problem, the project is transferred to small animals whereby advantage is gained in time and expense that would of necessity be great if the study was continued with large animals. When the problem has been solved by use of the laboratory subjects the findings may be applied to the original farm animal for final verification.

In an extensive series of sheep experiments conducted at the University of Alberta, symptoms presumably due to a nutritional deficiency were secured, but the specific nutrient that was deficient could not be determined from the data. Therefore, and in keeping with what has been outlined as the indirect procedure, it was deemed advisable to continue the study upon laboratory animals. To do this, however, it was necessary to place groups of these subjects on similar rations to determine the response of different species of small animals.

There is much evidence that the reaction of small animals to certain deficiencies is not uniform with that of farm animals either in extent or in the order of symptom appearance. The variety of symptoms associated with vitamin A deficiency was shown by the experiments of Hughes, Aubel and Lienhardt (1). These

workers state that in the rat the eye lesions, essentially corneal ulcers, primarily characterized the outward avitaminosis disorder, while in pigs a nervous disorder constituted the outstanding characteristic. It was noted, however, in extremely aggravated deficiencies in swine that corneal ulcers did ultimately appear. Nelson et al (2) found that rabbits, like chickens, showed both the marked eye lesions and symptoms of nerve degeneration in advanced stages of avitaminosis A.

Animals likewise vary in their ability to utilize certain essential substances. Ahmid and Malik (3) observed that rats, chickens, rabbits, pigeons and cats convert carotene into vitamin A with decreasing efficiency in the order stated, on an arbitrary scale as follows: 100, 24, 16, 1.2 and 0 respectively. Bills and co-workers (4) in testing a tuna fish liver oil for vitamin D potency report that chicks gave only one-sixth of the response recorded for rats. While these workers concluded the apparent presence of more than one vitamin D potency factor, the ability of the two animal organisms to utilize the composite oil was undoubtedly different. Riddell et al (5) state that they were unable to develop symptoms of marked aphosphorosis in rabbits, assuming, as had Australian workers, that rabbits apparently have a lower phosphorus requirement or else utilize this element in their feed to better advantage.

Therefore, before the problems arising from the sheep experiment could be studied with small animals, it was necessary to establish what species of small animal would be most suitable as experimental subjects.

SHEEP STUDIES

Before reporting the experiments conducted with small animals, it is necessary to understand the results obtained in the sheep feeding trials out of which problems arose that were believed possible of solution with the smaller animals.

Statement of the Sheep Problem.

During the period between November 1929 and June 1934, several sheep experiments were conducted in which different groups of ewes were maintained on different rations to study nutritional factors affecting wool growth and quality. It was noted that when breeding ewes were maintained on the commonly-fed non-leguminous hay and oat rations for long periods of time, six months and longer, they underwent a gradual loss in thrift as a result of poor appetite, subsequently their reproductive capacity was greatly reduced and their wool cover was inferior both quantitatively and qualitatively. Groups of sheep maintained on a high nutritional plane as well as those receiving protein (casein) and mineral (Ca, P, I and S) supplements, developed the symptoms previously described.

During the course of these trials a representative of the Canadian Co-Operative Wool Growers' Association* examined some of the ewes that had become unthrifty. He noted the similarity of the symptoms of the experimental ewes to those that developed in range ewes that were forced to exist upon naturally cured pasture throughout the course of exceptionally long winters.

Thus, the syndrome was accepted as due to some nutritional

---

*W. S. Benson, Field Representative for Alberta and British Columbia, Canadian Co-Operative Wool Growers' Association.

insufficiency and subsequent experiments were undertaken to determine the specific cause of malnutrition of the ewes on non-leguminous rations.

Deficiency Symptoms.

The general appearance was one of unthriftiness or malnutrition. Closely associated with this general observation were specific symptoms, notably, anorexia, loss in body weight, impaired reproduction and anaemia. It should be pointed out, however, that even among animals of the same group, thereby receiving similar treatment, there was a marked difference in the time and in the extent to which the deficiency was manifest. This indicates considerable individual variation.

Since ewes fed on a ration of alfalfa and oats did not develop any of the undesirable symptoms, the data for these ewes were used as a comparative standard in the analysis of results.

The recorded symptoms indicative of a nutritional deficiency, namely, anorexia, loss in body weight, impaired reproduction and anaemia, are discussed in greater detail in the following paragraphs.

Loss of appetite. Experimental ewes in both leguminous and non-leguminous lots were fed roughage supplemented by ½ lb. of ground oats daily per ewe, except during lactation, at which time the concentrate was increased. All roughages were fed ad lib but no waste was allowed in the alfalfa fed group, while a weigh-back was allowed in the non-leguminous group. From weigh-backs of less than 4 percent at commencement there was a rapid increase in volume of weigh-back coincident with a rapid appetite decline

till values of 33 percent and over were recorded. Both rations supplied total digestible nutrients and digestible crude protein in quantities more than sufficient to adequately provide for maintenance and pregnancy as proven by the work of Bowstead and Dowell (6). In both lots the concentrate was eaten with apparent relish. While alfalfa fed groups maintained a satisfactory level of roughage consumption throughout the experimental period, the oat and prairie hay groups did not, as shown in Table I.

TABLE I

INTAKE OF ROUGHAGES AND CONCENTRATES WITH CORRESPONDING TOTAL DIGESTIBLE NUTRIENTS

Date	Leguminous Lot lbs. per ewe-day			Non-Leguminous Lot lbs. per ewe-day		
Av. for period	Roughage	Conc.	Total T.D.N.	Roughage	Conc.	Total T.D.N.
Nov. 7	2.86	0.50	1.80	2.34	0.50	1.48
Dec. 5	2.86	0.50	1.80	2.40	0.50	1.50
Jan. 2	2.86	0.50	1.80	2.50	0.50	1.55
Jan. 30	2.86	0.50	1.80	2.48	0.50	1.55
Feb. 27	2.86	0.50	1.80	2.47	0.50	1.54
Mar. 27	2.78	0.50	1.76	2.47	0.50	1.54
Apr. 24	2.75	0.50	1.74	2.47	0.50	1.54
May 19	2.83	0.51	1.78	2.50	0.51	1.56
June 21	2.86	0.92	2.10	2.54	0.71	1.70
July 17	2.86	0.92	2.10	2.48	0.71	1.67
Aug. 14	3.43	0.86	2.34	2.67	0.71	1.73
Sept. 11	3.16	0.85	2.20	2.20	0.71	1.50
Oct. 9	3.00	0.80	2.08	1.78	0.68	1.31
Nov. 6	3.00	0.50	1.87	1.50	0.76	1.24
Dec. 4	3.00	0.50	1.87	*		

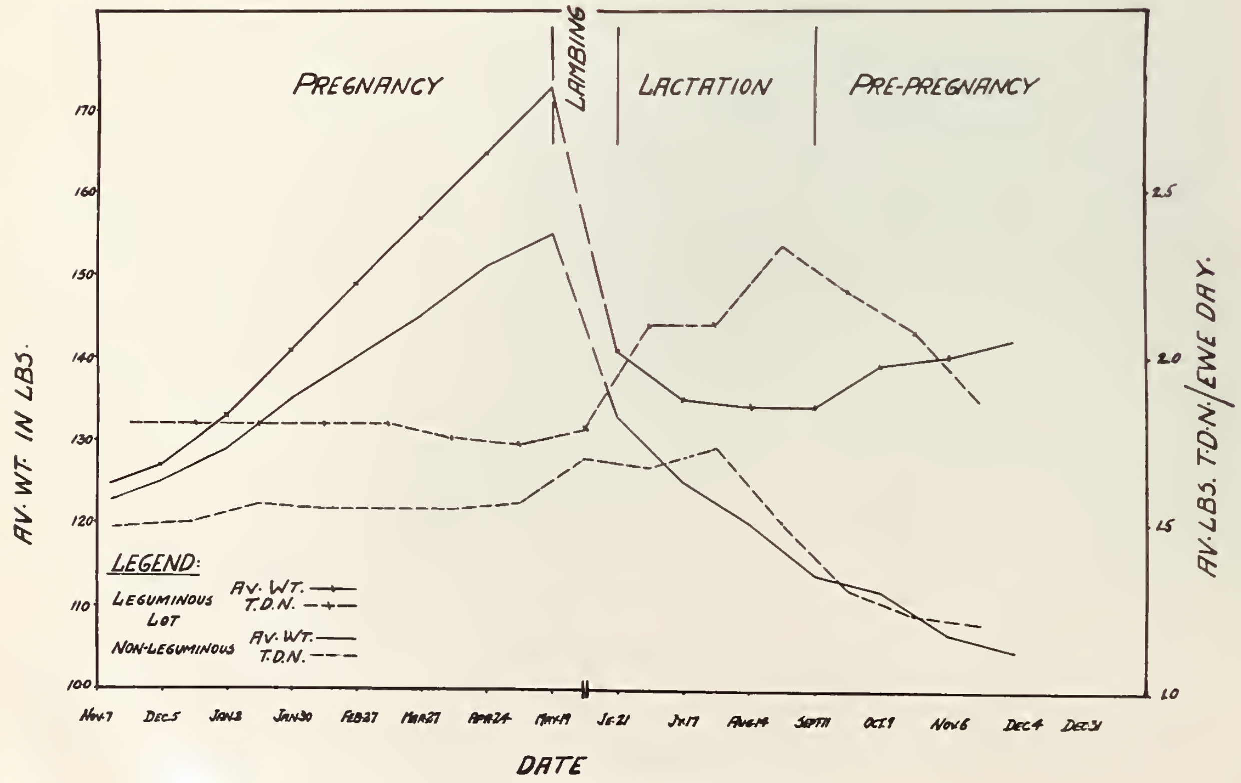
*Discontinued owing to extreme unthriftiness on Nov. 15.

Note - T.D.N. calculated according to MORRISON'S FEEDS & FEEDING - Table I.

The lower initial intake in the non-leguminous roughage group may have been the result of lower palatability of the oat and prairie hays, but the rapid and progressive decline in appetite after a

GRAPH I

Average Daily Ingestion of Total Digestible Nutrients and Corresponding Average Weights of Ewes on Leguminous and Non-Leguminous Rations.
(on page ~~10~~ 7)



period of 7 to 10 months was undoubtedly due to other causes. The increased roughage consumption during late pregnancy and lactation was an indication that appetite was not affected at that time. However, following the cessation of lactation, there was a progressive anorexia which necessitated the use of increased quantities of concentrate. Despite this adjustment, the rapid decline in roughage ingestion during the 10th, 11th and 12th months on experiment (August, September, October and November), resulted in a sub-maintenance level of total digestible nutrient intake. On November 15th the ewes were removed from the experiment and alfalfa hay was fed to remedy the deprived condition. While palatability of the oat hay - prairie hay mixture may have been a factor in decreasing consumption, a study of additional data suggests that the low roughage intake subsequent to lactation must have been due to some nutrient deficiency of the ration.

Loss in body weight. Normal body weight was maintained as long as the ewes continued to consume sufficient roughage. Upon the development of anorexia, body weight rapidly decreased as shown in GRAPH I.

Both leguminous and non-leguminous groups were placed upon experiment at the beginning of their pregnancy period and made gains during pregnancy that were consistent with the weight and number of fetuses. The average post-lambing weights in both lots were above those recorded as breeding weights but the difference [illegible] was not significant. Both groups lost in average body weight during the early part of the lactation period. The daily allowance of concentrate was increased as a compensatory measure. Despite this, the ewes fed oat-prairie hay roughage continued to lose weight even though they only weaned a lamb crop of 71 percent as compared with 157 percent for the alfalfa fed ewes. After weaning the latter group of ewes made rapid recovery. On the other hand, the non-leguminous group definitely showed a loss of appetite for roughage and their average body weight reached an extremely low level, 13.7 pounds below the initial weight of November of the preceeding year. At the same time, the alfalfa fed ewes recorded an average gain of 17.8 pounds even though the alfalfa roughage had been limited to 3.0 pounds/ per ewe daily for the three months preceding.

The graph also shows that there was a close correlation between body weight and the total digestible nutrients ingested particularly in the non-leguminous fed ewes after apparent appetite failure. It could be assumed, therefore, that their loss in weight and general unthriftiness were brought about by their failure to consume sufficient food to meet requirements.

Impaired reproduction. Although at the time of parturition the weight and thrift of the ewes and their lambs were not significantly

different, those ewes fed non-leguminous rations failed to suckle their lambs to weaning time as well as did the ewes fed alfalfa as is shown in TABLE II.

TABLE II

COMPARATIVE REPRODUCTION DATA OF EWES FED LEGUMINOUS AND NON-LEGUMINOUS RATIONS.

Ration	No. of Ewes	No. of Lambs	% Lamb Crop	Wt. Lamb Crop	Av. Wt. lbs. Lambs	Twins-	Singles	Lamb Crop Loss	No. Lambs Weaned	% Wear -ed Lamb Crop
Non-leguminous roughage plus oats	7	12	171	99.7	8.3	10	2	58%	5	71
Non-leguminous hay plus oats, tankage, linseed oil meal	7	8	114	78.9	9.9	2	6	25%	6	86
Alfalfa hay plus oats	7	13	186	108.4	8.3	12	1	15%	11	157

The cause of the higher lamb mortality in the non-leguminous lots was probably lack of milk. The growth of the lambs and the mammary development of the ewes suggested that at least in some cases the ewes failed to provide sufficient milk immediately after parturition or ceased to secrete milk before the offspring were old enough to subsist on other foods. Insufficient milk secretion on the part of the ewes fed non-leguminous roughage may have been the result of the unthrifty condition brought about by their low feed consumption or some nutritional deficiency or both.

Anaemia. Periodic haemoglobin determinations were made upon the bloods of some experimental ewes in the 1935 trials because the

unthrifty ewes in the previous experiment had shown marked symptoms of anaemia.

In the following table it will be noted that while a seasonal trend occurred in all lots there was no significant difference in the amounts of blood haemoglobin of ewes from the leguminous and non-leguminous lots.

TABLE III

BLOOD HAEMOGLOBIN OF EWES FED LEGUMINOUS AND NON-LEGUMINOUS RATIONS IN GRAMS

Date	Lot I Non-leguminous oat and prairie hays + oats		Lot II Non-leguminous oat and prairie hays + oats, + calcium, + phosphorus		Lot III Leguminous alfalfa hay + oats	
Jan. 24, 1935.	10.7	(4)	10.40	(2)	10.40	(2)
May 5, "	9.46	(4)	7.00	(2)	7.42	(2)
July 8, "	7.49	(3)	6.60	(2)	7.89	(2)
Sept. 7, "	7.98	(1)	7.47	(2)	7.98	(2)
ALL EWES Sept. 7 "	7.09	(9)	7.40	(9)	7.70	(8)

(x) Shows number of ewes tested.

Ewes that were used in this experiment had been on good brome pasture and therefore when allotted to their respective groups showed no significant difference in the amount of haemoglobin. The marked reduction in the amount of haemoglobin as indicated by the May analyses may have been the result of dry feeding, but more likely due to a condition known as "pregnancy anaemia" as described by Von Donk et al (7) and others (8). July determinations suggest that the first part of the lactation period may also have depressed the blood haemoglobin of ewes on basal rations while the blood

values of ewes on alfalfa rose to correspond more closely to the normal level. In September when blood haemoglobin of all ewes on experiment was determined no significant difference was evident. However, there were several animals in Lot I with extremely low haemoglobin levels.

The reason for lack of significance in the results may have been due to the fact that none of the ewes selected for haemoglobin determination were sufficiently unthrifty at the time of the test. Those that died or were removed may have shown low haemoglobin levels before death or removal.

In 1937 two unthrifty ewes from non-leguminous lots and one thrifty ewe from the alfalfa lot were slaughtered for detailed examination. Samples of blood were also taken for clinical blood study. The gross findings of the post-mortem revealed abnormalities in the unthrifty non-legume fed individuals as shown in Table IV. Low blood phosphorus, low blood albumin, low non-protein nitrogen and low blood haemoglobin were particularly significant. Based upon these findings a diagnosis of primary anaemia, microcytic in type, associated with some haemolytic process and with improper protein synthesis was given.

TABLE IV

POST-MORTEM FINDINGS IN EWES FROM LEGUMINOUS AND NON-LEGUMINOUS LOTS

Condition	Sheep No. 1	Sheep No. 2	Sheep No. 3
	Non-leguminous		Leguminous
	Very unthrifty	Unthrifty	Thrifty
Phosphorus	2.3 mgm. %	4.1 mgm. %	5.1 mgm. %
Albumin	3.00%	3.99%	4.35%
Globulin	3.37%	3.26%	3.21%
Non-protein nitrogen	36 mgm. %	37.5 mgm. %	48 mgm. %
Red blood cell count	8.1 million	11.09 million	13.4 million
Haemoglobin	7.09 gms.	10.5 gms.	11.15 gms.
Packed cells	17.9%	37.3%	40.3%
Bilirubin (Indirect Van Den Bergh)	0.4 mgm. %	Trace	Trace

*Other routine tests relatively normal.

It is believed, however, that the abnormalities revealed in Table IV could easily be secondary and the direct result of anorexia and inanition.

Therefore, in summarizing the observed symptoms associated with the unknown deficiency in the dry non-leguminous rations for breeding ewes, the following are characteristics:-

(1) Appetite failure after 7 months of dry feeding.
(2) Subsequent rapid decline in body weight.
(3) Impaired reproduction as the result of post-natal mortality.
(4) Anaemia, probably due to the effect of inanition.

The Use of Supplements in Past Studies on Sheep.

In the course of this series of experiments various forms of supplemented rations were compared against the basal deficient to determine the nature of the insufficiency. It has been explained previously that under a feeding programme where alfalfa hay was used as the sole roughage, the deficiency symptoms did not appear and all physiological processes within the body functioned normally. The problem then resolved itself into one of isolating the nutrient responsible, one that was present in alfalfa hay and lacking in non-leguminous roughages. Numerous supplements were fed in the belief that they might balance the non-leguminous ration.

Protein supplements. It was felt that the lower protein intake of the ewes in the non-leguminous lots may have been the cause of the development of unthriftiness. While these ewes consumed sufficient protein for maintenance during the first part of the trial, the protein may have lacked in quality.

First casein and later a tankage-linseed oil meal mixture was used. These supplements gave no curative effect although the appearance of acute deficiency symptoms was delayed for some weeks.

The symptoms were pronounced at the end of 7½ months on basal rations, while 9½ to 10 months were required for the group receiving the protein supplement.

While the difference in numbers of lambs born cannot be regarded as the result of differences in the ration, the progress of the lambs after birth was significant. The addition of protein to the basal ration apparently was beneficial during lactation as indicated by the lower post-natal mortality. The lamb crop in the groups which had received tankage and linseed oil meal in addition to the basal ration weighed only 78.9 lbs. as compared with 99.7 lbs. for the non-supplemented group. While the supplements used above depressed the lamb crop, they did provide for stronger lambs and better milk flow as indicated by a 25 percent lamb loss after parturition as compared with 58 percent for the basal group. Thus the extra protein undoubtedly served to lessen materially the strain of lactation and likewise the strain upon the body of the ewes as a whole. Since the ewes fed protein supplements ultimately did become unthrifty, it can be concluded that while the basal ration was apparently deficient in protein, an additional deficiency was also present that caused the development of symptoms indicating malnutrition.

Calcium, phosphorus and iodine supplements. The significantly lower calcium content of cereals, the lower phosphorus content of some of the common grasses, and the prevalence of goitre in Alberta suggested that the use of supplements of calcium, phosphorus and iodine with the basal deficient ration might prove beneficial.

Experiments have shown that animals fed rations deficient in any one of these minerals developed symptoms similar to those that

were observed in ewes fed the basal non-leguminous ration. A calcium deficiency is denoted by unthriftiness in young, particularly prior to the development of rachitic symptoms (10). Eckles (11) has proven that mature animals fed insufficient phosphorus develop an unthrifty condition, undergo emaciation and suffer from anorexia. In Michigan experiments the most prominent symptom of phosphorus deficiency was the latter (12). At the University of Alberta the feeding of potassium iodide to pregnant ewes improved the strength and weight of the lambs at birth (13).

Several groups of ewes have been placed on trial during 7 years of experimentation that were fed calcium, phosphorus and iodine supplements with the deficient non-leguminous ration. The feeding of these supplements to ewes on oat plus prairie hay and oat ration while not materially improving thrift and reproduction, appeared to benefit both ewes and lambs to some measure. At no time did these supplements stimulate a reaction on the part of the ewes as completely satisfactory as that given by alfalfa hay as a roughage. It is to be concluded that while calcium, phosphorus and iodine supplements did counteract slightly the nutritional deficiency of the basal ration, they failed to prevent the development of characteristic symptoms of malnutrition.

Iron and copper supplements. The anaemic condition of the ewes that were fed the non-leguminous basal ration suggested the possibility of an iron deficiency. Likewise Keith (14) has shown that a low iron diet is unfavorable for the most successful reproduction in rats. Since the effect of iron deficiency bore a relationship to the symptoms observed in the ewes on basal rations, iron chloride supplemented by a small quantity of copper sulphate

was fed.

A very poor lamb crop was produced. In the 1935 trials 68 percent of the lambs were born dead or very weak. Of the lambs that were born alive, 67 percent died later, leaving a net lamb crop of 33 percent as compared with 133 percent for the alfalfa fed ewes. The ewes fed iron and copper supplements were the first to become unthrifty in this set of trials. At first the emaciated appearance and poor reproduction was attributed to the nutritional deficiency of the ration, but later it was believed to be the result of continuous copper dosage which is toxic in effect as shown by Texas experiments (15).

Vitamin A and D supplement (cod liver oil). Symptoms of unthriftiness and low reproductive ability similar to those noted in the course of the sheep studies had been described in recent literature pertaining to a deficiency of vitamin A. Hart and Guilbert (16) in studying the abnormal condition developed by range cattle on parched range pasture reported that the manifestations are commonly limited to reproductive failures toward the end of the dry feeding season. Meigs (17) in reporting the results of calcium metabolism studies, states that when timothy hay and straw were fed to reduce the calcium intake, the resulting failures in reproduction and thrift are due to vitamin A rather than calcium deficiency.

The work of Huffman (18) indicates that symptoms as anorexia, general unthriftiness, abnormal blood analysis and low inorganic blood phosphorus are associated with a vitamin D deficiency in young cattle. The similarity of these symptoms to those of the deficiency under study, suggested that vitamin D may have some

bearing upon the insufficiency of non-leguminous rations for breeding ewes.

Therefore, in order to determine whether or not vitamin A or vitamin D was responsible for the deficiency, total or in part, the ewes fed basal rations plus calcium-phosphorus supplement were given in addition a good quality cod liver oil.

While the feeding of supplements of calcium, phosphorus, iodine and cod liver oil had significantly beneficial results, the performance of these ewes was not equal to the performance of those fed alfalfa hay in the maintenance of weight or reproductive ability. It would appear then, that alfalfa must contain an additional nutrient or nutrients not contained in the above supplemented basal ration.

Wheat germ meal. It is generally recognized that the germ of a plant seed contains a complexity of life-promoting substances and nutrients. It has, however, been definitely established that the germ of the seed contains vitamin E and that at least certain species of animals require this vitamin for reproduction. The studies of Evans and Bishop (19) which have since been confirmed by many investigators, showed that vitamin E is essential for normal reproduction and that wheat germ meal is a potent source of this vitamin.

Thus, in the 1936 trials the basal ration was supplemented with wheat germ meal. Instead of the usual ½ lb. of ground oats daily, the ewes received a concentrate mixture of 0.3 pounds of oats and 0.2 pounds of wheat germ meal per ewe daily. This supplement gave no improvement over the non-leguminous basal ration. Both supplemented and unsupplemented groups had commenced the experiment at an average per ewe weight

of 121.5 pounds in November. In August of the following year ewes in both lots were removed from the experiment due to extreme unthriftiness at an average weight of approximately 108 pounds, a loss of 13.5 pounds over the period. Characteristic symptoms of anorexia and impaired reproduction were common in both groups and in addition one ewe in the supplemented group receiving wheat germ meal died. It was concluded, therefore, that wheat germ meal did not provide the nutrient that was deficient in the basal ration.

From the results obtained by the feeding of various supplements, it was concluded that while the beneficial effects of some indicated certain specific deficiencies of the basal ration, none of the supplements enabled the ewes to maintain weight or reproduce as well as the ewes on alfalfa roughage. It is apparent, therefore, that the deficient factor is present in alfalfa and lacking in non-leguminous roughages and that it is not contained by any of the supplements discussed above.

Conclusions.

(1) A definite nutritional deficiency exists when breeding ewes were maintained upon oat and prairie hay roughage fed ad lib together with ½ lb. of oats daily.

(2) The characteristic deficiency symptoms become apparent after 7 months of dry feeding on basal rations as anorexia, gradual emaciation, impaired reproduction and anaemia.

(3) The deficiency is not one of protein, calcium, phosphorus, iron, copper, iodine or vitamins A, D or E, though the use of some proved beneficial in delaying the appearance of symptoms.

(4) The difficulties that have been encountered in seeking the specific insufficiency of the basal diet has suggested that smaller animals may be more suitable subjects upon which to continue the study of this problem.

RAT STUDIES.

Introduction.

The albino rat is the most common laboratory animal used in nutritional research work. Because of their genetic uniformity they have responded uniformly to known nutritional deficiencies. For these reasons the rat was used for the purpose of studying a ration known to be deficient when fed to breeding ewes.

Preliminary.

In the fall of 1936 the Department of Biochemistry very generously made available a sufficient number of young female rats to make up three groups, each of which was to be fed a different ration. Since it was necessary to check the experimental rations with ones that we believed to provide adequate nutrition for rats, a number of recognized rations were carefully studied including the University of Alberta basal. It was found that these stock rat rations used by nutritional authorities vary to some considerable degree in the feeds used and the crude nutrient content. However, both the Steenbock and the Pennsylvania State College stock diets have been used as basals for many rat experiments (20) and (21) with every indication that sufficient essential nutrients were supplied for normal reproduction and growth. However, data on the growth and reproduction of rats for these two rations were

not available.

The University of Alberta basal formulated by Drs. G. Hunter and W. D. McFarlane, has been in use for several years as a complete ration. Reproduction records were available and thus were used in evaluating the data of the trial being reported.

The ingredients of the three stock diets mentioned, together with their respective proportions therein are as follows:

The Steenbock Rat Ration (20)

Yellow corn	76%	Ground alfalfa	2%
Linseed oil meal	16%	NaCl	0.5%
Crude casein	5%	$CaCO_3$	0.5%

Butter - 5% of above weight

This ration is supplemented by an occasional feeding of lettuce.

The stock diet as fed to rats at the Institute of Animal Nutrition of the Pennsylvania State College (21) is as follows:

Yellow corn	25%	Blood meal	10%
Wheat Red Dog flour	22%	Ground malted barley	10%
Oat flour	15%	NaCl	1%
Linseed oil meal	15%	$CaCO_3$	1%
		Bone meal	1%

Wheat germ middlings - 5%) added to the
Cod liver oil - 5%) above by weight

A small dose of wheat germ oil was administered daily to each pregnant female.

The University of Alberta Stock Ration (22)

Wheat	59.22%	Casein	3.89%
Linseed oil meal	12.40%	Alfalfa meal	1.55%
Whole milk powder	22.07%	NaCl	0.39%
		$CaCO_3$	0.39%

Cod liver oil, carrots) lettuce) muscle meat, the latter in very limited quantities after littering.

A crude nutrient composition of each of the three rations has been calculated from analyses data in "Feeds & Feeding" by

Morrison (23) and in a comparative table appears as follows:

TABLE I.

THE CRUDE NUTRIENT COMPOSITION OF THE THREE STOCK RATIONS

	Cr.Protein	Cr.Fat	Cr.Fibre	N. Free Extract	Mineral
Steenbock	17.03%	7.67%	3.33%	56.6%	3.15%
Pennsylvania	23.87%	9.06%	3.95%	48.8%	5.34%
Alberta	22.39%	8.29%	2.77%	54.7%	4.11%

Since the Steenbock and Pennsylvania rations have proven adequate for growth and reproduction, and since the feeds used and composition of the Alberta ration are similar to the average of the other two, it may be assumed that the Alberta ration was also adequate.

There is no evidence to indicate that the white rat is able to grow and reproduce normally on a ration which is high in fibre. Hathaway, Davis and Graves (20) in carrying out experiments on the vitamin E content of field-cured alfalfa hay, used a ration containing approximately 5.6 percent of fibre when 20 percent alfalfa hay was used in the ration. An extract from their results is as follows:

"Since as little as 3 percent of field-cured alfalfa was sufficient to supply enough vitamin A for good growth, and since 20 percent alfalfa produced good reproduction, it seems that a shortage of these vitamins would never be responsible for failure in growth or reproduction in livestock having a good grade of field-cured alfalfa included liberally in the ration. The above statement is made on the assumption that farm animals respond

similarly to rats." This may not be the case when consideration is given to their type of digestive systems. Being a rodent, the rat has an alimentary tract not unlike that of the pig, and for that reason it seems logical to suppose that rations high in fibre would be inefficiently used in both cases. Insofar as the growth of the pig is concerned, Evvard (24) concluded that the depressing effect of each additional percentage of fibre in the ration of 2% to 5% is small but quite marked over 5%. Hence the maximum fibre content of a rat diet should most likely be within the same range from the standpoint of growth. Evidently fibre is not a factor in reproduction provided all essential nutrients are supplied in sufficient quantity (20).

Experimental Procedure.

The experiment was started upon three groups of albino rats (Mus Norwegicus Albinus) designated as Lot I, II and III, but the basal group Lot I on the University of Alberta stock ration was discontinued. The females in this group were not similar to the others, they were twenty-three days older and hence were considerably heavier in weight; some had been bred previous to commencement of the experiment and finally, after they had shown adverse reaction to experimental routine, it was felt that these females would not contribute data of suitable comparative nature. All rats were the progeny of females of Wistar breeding, but having been chance-mated for many generations, were highly outbred. Those of Lot II and III, nine females in each, were the weanlings of three litters, evenly distributed as to numbers.

Hence all experimental females were the same age (123 days), the same average weight in each of the two lots (184 grams) and in all cases were to be bred and consequently to litter for the first time.

The females of both groups from weaning to commencement of the experiment had been fed on the University of Alberta stock ration and hence all were nutritionally uniform. From the time that the experiment was started, Lot II rats were placed on a ration, designated as (A), which was similar to the University of Alberta stock ration except that the alfalfa hay was replaced by an equal quantity of oat hay and timothy hay. Lot III ration, indicated as (B), was quite unlike the above (A). From the standpoint of chemical composition, it was designated to conform fairly closely to accepted rat rations except from the standpoint of fibre. This constituent was practically doubled. As to other ingredients, they were selected from feeds commonly used in sheep rations. The necessary crude protein, minerals and carbohydrates were supplied in the form of starch, sucrose casein and standard salts. The two rations under study are as indicated below:

Lot II - A:	Wheat	59.20%	Oat hay	0.78%
	Whole milk powder.	22.07%	Timothy hay.	0.76%
	Linseed oil meal .	12.40%	NaCL	0.39%
	Casein	3.89%	$CaCO_3$	0.39%

Cod liver oil ± ½ cc. per rat daily.

It was calculated that the above ration contained 22.2% crude protein; 8.2% fat; 2.9% crude fibre; 54.8% nitrogen-free extract and 4.04% mineral matter.

Lot III - B:	Oats	20.00%	Casein (crude) .	17.17%
	Oat hay	8.01%	Sucrose	6.01%
	Timothy hay	7.01%	$CaCO_3$	0.50%
	Corn starch	41.04%	NaCl	0.50%

Cod liver oil ± ½ cc. per rat daily.

Calculation revealed the following composition: Crude protein, 17.4%; fat, 1.3%; crude fibre, 6.5%; nitrogen-free extract, 65.5%; and mineral matter, 3.2%.

All feeds were finely ground, thoroughly mixed and fed as a dry mash ad lib from open containers. The cod liver oil was worked into the mash at the rate of ±½ cc. daily per rat. Each lot of females was confined in a large 2' x 2' x 2½' - No. 9 wire cage and at such a time as the females showed evidence of heavy pregnancy they were transferred to individual littering pens.

Beginning on the first day of experiment, each member of each lot was weighed every third day. After the litters had arrived, weighings on these were made with their respective dams, thus giving consecutive three day weights on all females for the period of the experiment and on litters till weaning. In all cases the pups were weaned at the age of 21 days and at such a time the females were returned to their respective cages for breeding.

Three vigorous males were kept in each of the main cages at all times. These were rotated in nine day periods through a special "pep-up" cage where they were fed the University of Alberta stock ration together with greens, wheat germ meal and carrots. This procedure was adopted to eliminate or at least equalize any deficiency imposed upon the males while in the breeding cages of Lot II and Lot III and in that way avoid any possible sterility in the males.

Results.

Since the major purpose of this study on rats was to determine whether or not this species would develop characteristic

symptoms of unthriftiness and low reproductive ability, the recording of feed consumption was not regarded of sufficient importance to warrant its inclusion.

Before presenting the data secured, mention should be made concerning features which caused variability in the data obtained during the course of the experiment. Fright, irritability and instinct were the general reaction to constant handling after commencement of the study. Consequently, after littering, such vices as litter destruction at birth, lack of maternal instinct and crushing became evident and, as is frequently the case, the above mentioned vices took the form of habits in the case of certain females. Though this situation for the most part cleared itself after the first litters had been born, certain measures of variability of data ensued, thereby reducing the significance of the results. When, however, these abnormal results which did not appear to be due to the diet are not included in the analysis of results, more uniform data remained and the effect of the diet became more evident.

Reproduction. In order to evaluate the reproductive performance of all females in Lot II and Lot III, average figures for the number of pups littered and the number weaned for the stock female rats at the University of Alberta rat colony were used for comparison.

TABLE II

REPRODUCTIVE PERFORMANCE ON THREE DIFFERENT RATIONS.

Preg.		Check	No. litters	Lot II	No. litters	Lot III	No. litters
1st	Total No. pups littered	341	38	60	7	61	7
	" " " weaned	257	45	20	7	11	7
2nd	" " " littered	341	38	52	7	27	5
	" " "" weaned	257	45	39	7	9	5
3rd	" " " littered	341	38	38	5	19	3
	" " " weaned	257	45	27	5	9	3

Calculated as direct averages of data from Table II, the average numbers of pups littered and weaned are as follows:

TABLE III

AVERAGE NUMBER OF PUPS LITTERED AND WEANED ON THREE DIFFERENT RATIONS.

Pregnancy		Check		Lot II		Lot III	
1st	Av.No.pups littered	8.87±0.2		8.57±0.8		8.4±0.9	
	" " " weaned	5.71±0.5	64%	2.50±1.2	29%	1.4±0.6	17%
2nd	" " " littered	"		7.43±0.7		6.0±0.6	
	" " " weaned			5.57±3.7	75%	1.3±0.9	22%
3rd	" " " littered	"		7.5 ±0.8		6.02±0.6	
	" " " weaned			4.5 ±1.9	60%	1.8 ±1.7	30%
AV.	" " " littered	8.87 1.2		7.83		6.80	
	" " " weaned	5.71 3.5	64%	4.29	55%	1.50	22%

The similarity of numbers littered among the stock and experimental females during the first pregnancy is significant. It would appear that any deficiencies in the rations of Lot II and III were offset by the storage of sufficient nutrients during the pre-experimental period while the rats were on the University of Alberta basal diet. Hence no symptoms of a nutritional deficiency appeared during the course of the 1st pregnancy period on rations II and III.

The reduction in the size of litters dropped by the females at the end of the 2nd and 3rd pregnancies would indicate that some nutritional deficiency did exist and also the extent of that deficiency. Table III shows that there was a reduction of one pup per litter in Lot II and 2.4 pups per litter in Lot III or a percentage reduction of 11.8% and 28.6% respectively.

Observations from data compiled by Donaldson (25) indicate that the number of individuals in the litter generally increases from the first to second litter and decreases thereafter.

1st litter - an average of 6.2 pups
2nd " - " 7.9 "
3rd " - " 7.1 "
4th " - " 6.4 "

Since no such increase of 1.7 pups per litter between the first and second litters and 0.9 pups between first and third litters were secured in these trials, it would appear that the rations fed to Lots II and III were decidedly deficient.

Interpretation of data regarding number of pups weaned is difficult because of the numerous related and interacting factors that were involved. As explained in the introduction to this discussion the reaction of some of the females to experimental routine was quite abnormal and may have resulted in a subsequent recurrence in the form of habit. The extremely low percentage of weaned pups from the first litters in both Lot II and Lot III was no doubt due to factors associated with "handling." Litter destruction occurred frequently during the first pregnancy but became less marked in later pregnancies which did much to raise the percentage weaned. It is extremely interesting to note, however, that once adjustment to the new environment did take place the number of pups weaned by females in Lot II compared very favorably with those of the check group, whereas Lot III females continued to destroy their young despite identical treatment. The lower number of pups weaned for the third litters in Lot III cannot be attributed to more killings. There is every indication

that some deficiency in the ration of Lot III females accounted for the greater number of killings or deaths of pups in this group.

Losses in weight at parturition are definitely correlated with size of litters dropped. The data in Table IV would indicate that the females in Lot III cast smaller litters than those of Lot II which is in accordance with the figures in litter size and thus further indicates the greater deficiency of Lot III rations.

TABLE IV.

AVERAGE WEIGHT OF LITTERS PRODUCED AND AVERAGE TIME IN DAYS BETWEEN EXPOSURE TO BUCKS AND LITTERING.

Pregnancy	No. of Females		Av.Gms.loss in wt. at littering		Time in days between exposure to bucks & littering		Normal Gestation
	Lot II	Lot III	Lot II	Lot III	Lot II	Lot III	
1st	*8	'8	57.6	54.0	26	25.3	21 dys. 15 hrs to 22 dys. 15 hrs + 1 to 4 dys. for impregnation
2nd	**7	"7	51.2	44.0	28	45.4	
3rd	7	'"5	48.0	45.0	27.7	33.0	
Av.			52.3	47.7	27.2	34.6	

* One Lot II female when found to be completely sterile was destroyed.

** A Lot II female died when unable to litter.

' A Lot III female died when unable to litter.

" A second Lot III female became sterile after 1st litter cast.

'" Two Lot III females did not become pregnant for third time.

Normal reproduction includes ease of conception. The data presented in Table IV shows that the females in Lot II conceived more readily than those of Lot III.

The normal gestation period for a female rat is in the neighborhood of 22 days, while 1 to 4 days is required for im-

pregnation (26). With little variation the Lot II females have produced litters in an average of 27.2 days. On the other hand, in Lot III there was a wide variation in the time required. In addition, one female became sterile after her first pregnancy and to all appearances, based upon weight fluctuation, continued to resorb the fetuses after a short period of development.

Since the first litters were cast in 26.0 and 25.3 days for Lots II and III respectively, the impregnation and gestation periods may be regarded as normal for both groups. The stock ration upon which the females had been maintained previous to the commencement of study, no doubt provided for considerable storage of the essential nutrients necessary for normal reproduction. However, the time required to produce the 2nd and 3rd litters was 28 and 27.7 days in Lot II, while the Lot III females needed 45.4 and 34.6 days to complete similar reproductive functions.

Whereas Lot II ration may lack only mildly in some essential, the Lot III ration appeared decidedly deficient and it would indicate that in order to even promote conception and pregnancy a prolonged time was necessary for the storage of sufficient of the specific nutriment.

Observations during the experimental period were that the Lot III pups at time of birth were weaker than those of Lot II, which further supports the belief that the Lot III ration was more deficient than that of Lot II.

An increase over normal of 2.0 and 1.7 days for Lot II as compared with 20.0 and 7.7 days for Lot III, may be regarded as extremely significant. Only five Lot III rats were included in the third pregnancy because the two remaining females had taken

an unusually long time to become pregnant for the first and second time. This would indicate that the average figure for the third gestation is too low since the necessary time was not given for the inclusion of the above mentioned females.

Growth. If the poorer reproductive ability of Lot III was due to nutritional deficiency, either general, caused by low quantity of total digestible nutrients, or specific, attributable to some essential factor, one would expect that growth would also be impaired. But since fewer pups were littered and suckled, the nutritional requirements of the females in Lot III was markedly reduced and they should have been able to maintain their body weight more readily than the females in Lot II. The fact that the females in Lot III weighed an average of 30 grams less than those in Lot II indicates that the ration fed to Lot III was less efficiently utilized.

TABLE V.

AMOUNT OF FIBRE IN THE RATIONS AND FINAL WEIGHT ATTAINED BY FEMALES

	Am't.Fibre in ration	No. of females	Av.Original Wt.(Oct.9)	No. of females	Final Wt. Feb.12-13	% Increase
Lot II	2.9%	9	184 gms.	7	255 gms.	38.6%
Lot III	6.5%	9	184 gms.	7	225 gms.	22.3%

The rather high fibre content of Lot III ration, 6.5% as compared with 2.9% for Lot II, may in itself play some part in reducing the rate of growth since the amount present is somewhat above that usually included in stock rations. This assumption is based upon the studies made with swine in their failure to utilize

high fibre rations (24) as mentioned in an earlier section of this paper. Since Lot III females gained only 41 grams in the 127 days on experiment as compared with 71 grams for Lot II females, and since the former were not exposed to the same drain by reproduction and lactation, it is apparent that the average gains made by Lot II, being 1.73 times those of Lot III, are far nearer normal than are the average gains of Lot III females.

Further support is added to the significance of the above data by Table VI in which appears a comparison of the number of pup-suckling days.

TABLE VI.

NO. OF PUP-SUCKLING DAYS

Lot No.	1st Preg.	2nd Preg.	3rd Preg.	Av. No.
Lot II	52.5	102.4	113.4	89.4
Lot III	33.0	38.0	47.2	39.4

Despite the evidence that Lot II females have been subjected to a drain twice as heavy as that of Lot III, and it must be conceded that lactation does impose a retarding factor, they are still able to show far greater gains and therefore growth than the females of Lot III.

In summarizing the data presented regarding the growth of the females while on experiment, it appears that while the high fibre of Lot III may have played its part in reducing the rate of growth, other deficiencies, as intimated previously, have also markedly influenced the rate of gains.

Summary.

In this study of the reactions of female rats to two widely different rations, certain definite observations have been made. They may be summarized as follows:

I. The females of Lot II cast and weaned more pups than the females of Lot III.

II. Females of Lot II required less time to conceive after placing them with the bucks than did the females of Lot III.

III. The Lot II rats made greater weight gains while on experiment, even though they did produce and wean more pups.

Conclusions.

I. The ration that was fed to Lot III rats was decidedly more deficient than that fed to Lot II females. The inadequacy of Lot III ration may be due in part to its high fibre content, but since the amount of fibre contained is only slightly in excess of that in recognized stock diets, a specific nutritional deficiency is definitely indicated.

II. Provided that the fibre of the ration is adjusted to approximately 5%, female rats may be used to continue feeding experiments started with breeding ewes. It is felt, however, that another species of laboratory animal capable of utilizing greater quantities of fibre may be found more suitable because the nutritional differences of the experimental rations would be of more nearly the same magnitude as that fed to sheep.

RABBIT STUDIES

During the winter of 1936-1937 rats were used experimentally to determine their response to rations that were similar to those found deficient when fed to sheep. As rats are unable to utilize

rations as high in fibre as those fed to sheep (20), it was necessary to add considerable starch, sucrose and casein to lower the fibre to a suitable level. While the results indicated that rats were quite satisfactory for the continuation of studies with the deficient sheep ration, it was felt that another species of laboratory animal may prove more suitable because none of the purified nutrients would be used and they could be fed rations more similar to the sheep rations. For these reasons female rabbits were procured and subjected to experimentation.

Objects.

(1) To establish the value and suitability of domesticated rabbits as laboratory animals for substantiating the results of sheep feeding experiments.

(2) To compare the value of alfalfa hay and an oat hay - prairie hay mixture as roughages for breeding does.

(3) To determine the relationship, if any, of nutrition to the haemoglobin content of the blood of breeding does as indicated by the two rations under study.

(4) To study the accumulative effect of the non-leguminous ration upon the offspring of non-legume fed does.

Outline of Experiment.

Two lots of does were placed upon leguminous and non-leguminous rations respectively to study their response in feed consumption, body weight, reproduction and blood haemoglobin. Weighing, breeding and haemoglobin determinations were carried out simultaneously throughout the course of the experimental period. Upon weaning, the offspring from the first gestation were fed the rations of their mothers.

Rabbits used and their Allotment.

This experiment included twelve previously inbred spring born does that were unlike in size, color and to some extent breeding. All does were subjected to a two week feeding period on non-leguminous roughage and oat groats to offset the effect of previous nutrition. These animals were divided into two groups as evenly as possible with respect to age, condition and weight, and the allotment was made insofar as possible on a litter mate basis.

In addition, two groups of weanling rabbits from the first litters were continued on the same rations as their mothers to study their response and thereby the accumulative effect of the rations fed.

Management.

Each female was confined in an individual wire cage with mesh floor, 2.5' x 3' x 1' in size, and received hay, grain and water daily. The does were mated to one of two bucks shortly after commencement of the trial and rebred repeatedly one month after parturition till termination of the experiment. At time of littering, excelsior shavings were placed in the cages to permit the building of nests. The young were weaned at four weeks of age. After weaning the young of the leguminous and non-leguminous lots respectively were group-fed corresponding rations.

Rations and Methods of Feeding.

The two groups, designated as Lot I and Lot II, were fed leguminous and non-leguminous rations respectively, similar to those previously fed to sheep. All feeds were selected from

local sources, were of good average quality and insofar as possible corresponded to the feeds used in the sheep trials. An effort was made to have both groups consume equal quantities of total digestible nutrients, calculations being based upon comparative figures available in the 20th edition of "Feeds and Feeding," by Morrison, and upon analyses made of the feeds by the Provincial Analyst.

The does in Lot I were fed alfalfa and oat groats while those in Lot II were fed oat and prairie hays and oat groats. The daily allowance per doe was subject to adjustment from time to time to facilitate equalization of total digestible nutrients. The does were allowed all the roughage that they would consume and in addition oat groats were fed in quantities believed to make up the necessary total digestible nutrients for the entire ration.

Data Recorded.

All data believed to have a bearing on the solution of the problem were recorded.

Weights of animals. Weights of each experimental animal were taken on three consecutive days at the time of breeding and the average regarded as the breeding weight. Likewise, weights were taken on three consecutive days after parturition, the average being taken as the post littering weight. The periodic differences represent the respective gain or loss in weight during each pregnancy and lactation period.

The progeny groups were individually weighed periodically during the course of their experimental period.

Reproduction. The total number born, number of young dead,

number died and number suckled to weaning were recorded insofar as possible. Some litter weights were obtained at birth but for the most part the young were permitted to suckle for one month and were then weighed and weaned.

Blood tests. Sahli haemoglobin determinations were carried out when weights were taken. Similar determinations were made on the offspring on completion of their experimental feeding period.

Experimental notes. Notes were made on all abnormal conditions which were observed during the course of the experiment and which would appear to have a bearing upon the interpretation of the results.

RESULTS.

Feed Consumption.

The effect of the different rations upon feed consumption of the rabbits was studied because the important individual symptom observed in the ewes fed non-leguminous roughage was loss of appetite.

While each doe was fed individually, considerable difficulty was encountered in measuring the roughage wasted by each. A great deal of time was also saved by collecting the wasted and refused roughage for each lot and from these the amounts consumed were calculated bimonthly for each lot. These consumption figures were used in adjusting the amount of feed to be given during the subsequent periods to enable both lots to consume equal quantities of total digestible nutrients. The periodic consumption of roughages and concentrates in Lots I and II is presented in Table I.

TABLE I

AVERAGE DAILY FEED CONSUMPTION PER DOE IN LOT I AND LOT II IN GRAMS

	Lot I			Lot II+		
Period	Alfalfa	Oat Groats	T.D.N.**	Total Roughage Oat & Prairie Hays	Oat Groats	T.D.N.*
Nov.15-30	73.9	32.5	69.8	71.6	32.5	68.0
Dec. 1-15	101.8	25.0	77.9	100.3	40.0	90.2
Dec.16-31	99.9	29.4	80.9	96.8	26.9	80.4
Jan. 1-15	100.7	30.0	81.9	104.2	25.0	78.5
Jan.16-31	102.7	30.0	83.0	115.5	25.0	84.5
Feb. 1-15	97.8	23.3	74.2	99.9	23.3	74.6
Feb.16-28	94.1	21.5	70.6	101.9	21.5	74.0
Mar. 1-15	103.8	16.7	71.3	107.4	16.7	72.5
Mar.16-31	108.3	21.4	78.1	111.0	21.4	78.8
Apr. 1-15	118.4	32.7	93.9	119.0	32.7	93.4
Apr.16-30	102.5	30.0	82.9	102.1	30.0	81.1
Av.	100.4	26.6	78.6	102.7	26.8	79.6

+ One doe died in Lot II on the 3rd of March from causes not of nutritional origin.

** - * Re T.D.N. - Digestibility values taken from Morrison's "Feeds and Feeding" 20th edition.

While there were certain differences in the amounts of feed consumed by the two groups in any one period, the average feed consumption during the entire trial was slightly in favor of the does in Lot II receiving the non-leguminous roughage.

Lot II does individually consumed an average of 2.2% more roughage and 0.8% more oat groats per day than did the does in Lot I. At no time during the trial did the females on the non-leguminous ration show symptoms of anorexia which was so evident in the sheep trials on similar rations.

Body weight.

In the light of the slightly increased consumption of Lot II it would be expected that the does in this lot would show a slight

increase in live weight as compared to those in Lot I. This, however, was not the case as shown in Table II.

TABLE II

PERIODIC NON-PREGNANT WEIGHTS ON LEGUMINOUS & NON-LEGUMINOUS RATIONS

	Initial Wt. Nov. 12	Dec. 13	Jan. 27	Mar. 28	Final Wt. Apr. 30
Lot I	1920	2145.8	2428.8	2416.8	2613.0
Lot II	1920	2161.3	2415.0	*2217.0	*2474.0

* Adjusted to include doe that died on March 3rd.

The table shows that at the end of the trial the does in Lot II on the non-leguminous ration weighed an average of 139 grams less than the does in Lot I. Significant differences in the average weight between the two lots were not noted until March 28th when the does in Lot II weighed approximately 200 grams less than those in Lot I due in part to the fact that the does in Lot II had suckled 56% more young than the does in Lot I. During the pregnancy period between March 28th to April 30th, the females in Lot II gained 257 grams as compared with 197 grams in Lot I. The fact that the does in Lot I had carried less young than those in Lot II was apparently the deciding factor. It will be noted, however, in Table III that during the entire trial both groups of does produced practically the same number of young.

The difference of 139 grams in the final weights of the two groups is not significant in that considerable variability in the gains made by the does in each lot did exist. There is, however, an indication that the non-leguminous ration was less efficiently

utilized by the does than was the leguminous ration.

Reproduction.

The data pertaining to reproduction are limited and may have been influenced by external factors to such an extent as to reduce their value. The does were somewhat young when bred for the first time and when placed under artificial environment may not have responded normally. No pregnancies resulted from the first matings and later matings did not result in all the females becoming pregnant. Some does displayed a lack of maternal instinct by not preparing a suitable littering nest and by not permitting the young to suckle. The eating of young was also observed in both groups. No significance can be attached to data as numbers born, number died due to weakness, etc., except where immediate check was made after littering. The number of young weaned was by necessity directly affected by the behavior of the mother during lactation and, therefore, of no value except to measure the extent of lactation. These data appear in Table III.

TABLE III

THE EFFECT OF LEGUMINOUS & NON-LEGUMINOUS RATIONS ON REPRODUCTION

	Lot I	Lot II
1st Littering.		
No. of does bred	6	6
No. of does littered	5	5*
No. of young born	32+	30+
No. of young weaned	9	14
2nd Littering.		
No. of does bred	6	5**
No. of does littered	3	2
No. of young born	21+	9+
No. of young weaned	9	2
Total No. Weaned	18	16
Av. No. per doe bred	1.5	1.5

* One premature litter cast.
**One female died on March 3rd.

The 8 litters in Lot I and the 7 litters in Lot II were weighed when the count was taken. Lot I litters weighed an average of 337 grams as compared with 208 grams for Lot II. The average weight per young at birth in Lot I was 48.2 grams and 46.2 in Lot II.

Since the above data show no significant differences between the two groups it may be concluded that differences in the ration did affect reproduction.

Blood Haemoglobin.

Blood haemoglobin was determined for each female at the time weights were taken. A summary of these data appears in Table IV.

TABLE IV

THE EFFECT OF LEGUMINOUS & NON-LEGUMINOUS RATIONS ON BLOOD HAEMOGLOBIN.

	Nov. 13	Dec. 14	Jan. 15	Mar. 28	Apr.30
Av. Lot I	11.0 gms.	10.9 gms.	11.4 gms.	11.2 gms.	11.3 gms.
Av. Lot II	10.7 "	10.3 "	11.0 "	9.7 "	10.6 "
Difference	0.3 "	0.6 "	0.4 "	1.5 "	0.7 "
% Difference	2.8%	5.8%	3.6%	15.9%	6.6%

There was considerable variation in the percentages of haemoglobin within each lot. It was observed, however, that a slight positive relationship did exist between differences in blood haemoglobin and differences in live weight. On March 28th when the does in Lot II weighed on the average 200 grams less than those in Lot I their blood haemoglobin level was 15.9% lower. Differences in haemoglobin on other dates showed a similar tendency but were not

significant. A significant difference in the amount of blood haemoglobin was obtained in the subsequent feeding trials with the young.

Insofar as these trials are concerned, alfalfa did not show any marked superiority in maintaining the normal blood haemoglobin level as shown by the rapid recovery in this regard of Lot II does after cessation of lactation on March 28th.

Performance of the Progeny Groups.

The offspring of the first litters were weaned at one month of age and were placed upon the rations of their mothers to which whole milk powder was added to increase the total digestible crude protein and reduce the fibre content. Casein was added to the ration of the non-leguminous group to equalize the protein intake for both groups. The powdered milk was removed from the rations after two weeks and the experiment was continued for a further two weeks to give a total of 32 days on trial. Weights were taken periodically and haemoglobin determinations carried out at the conclusion of the trial.

Progeny weights. There were 9 and 14 offspring on leguminous and non-leguminous rations respectively. At weaning Lot I offspring were an average of 70 grams heavier as the probable result of better thrift at birth followed by a greater milk supply afforded by the does in this group during the suckling period. The progressive increase in weight for these two groups are presented in Table V.

TABLE V

PROGENY WEIGHTS ON LEGUMINOUS & NON-LEGUMINOUS ROUGHAGE.

Total Wt.	March 30	April 9	April 20	April 30
Lot I Leguminous	3045 gms.	4774 gms.	6630 gms.	7991 gms.
" II Non- "	3763 "	6007 "	7646 "	8586 "

Table V (continued)

Progeny Weights on Leguminous & Non-leguminous Roughage

	March 30	April 9	April 20	April 30
Av. Wt. per Animal:				
Lot I Leguminous	338.3 gms.	530.4 gms.	736.7 gms.	889.9 gms.
" II Non- "	268.8 "	429.1 "	546.1 "	660.5 "
% Increase in Av. Wt. per Animal:				
Lot I Leguminous		58.8%	38.9%	20.5%
" II Non- "		59.6%	27.3%+*	20.9%

+ One animal injured on April 15th which adversely affected gains.
* Another animal failed to make gains for unknown reasons.

During the 32 day feeding period the offspring in Lot I increased their average weight by 162.5 percent over their initial average weight. The comparable increase for offspring in the non-leguminous lot was only 145.7 percent. The periodic percent weight increases in the two lots were not significantly different except in the period between April 9th and April 20th as indicated in Table V. Lot I gained 38.9 percent over this 11 day period while Lot II gained only 27.3 percent. Two factors may have been responsible, namely, two individuals in Lot II failed to make satisfactory gains during this period, and secondly, the two groups were placed upon a lower plane of nutrition by removing the whole milk powder. The latter factor affected the available digestible crude protein of Lot II offspring to such an extent as to reduce growth. Excluding this accepted variation which may have been due to the proposed influences, it would appear that in both groups the percent weight increase or periodic growth is directly proportional to the initial weight.

Feed consumption. Again, as in the parent groups, the concentrate was fed in limited quantities while roughage was supplied ad lib. Salt was supplied to both groups and calcium carbonate and casein were fed in addition to the concentrate in the non-leguminous group to compensate for the higher calcium and protein content of the alfalfa roughage. In view of the uneven total weights of the animals in the two progeny lots and the difference in numbers, it was deemed advisable to feed concentrates proportionally to a logarithmic factor of the individual body weight of animals comprising the two groups since metabolic function of the animal body has been shown to be related to the 0.73 power of the body weight rather than to body weight (27). The amount of total digestible nutrients and digestible crude protein ingested periodically are shown in Table VI.

TABLE VI.

TOTAL DIGESTIBLE NUTRIENTS AND DIGESTIBLE CRUDE PROTEIN INTAKE PER UNIT AVERAGE BODY WEIGHT$^{(0.73)}$ IN GRAMS BY OFFSPRING ON LEGUMINOUS AND NON-LEGUMINOUS RATIONS.

Period	Lot I Leguminous Ration			Lot II Non-Leguminous Ration		
	Av.Body Wt.$^{(.73)}$	T.D.N.	D.C.P.	Av.Body Wt.$^{(.73)}$	T.D.N.	D.C.P.
	gms.	gms.	gms.	gms.	gms.	gms.
Mar.30-Apr.10	745.4	3.59	0.72	994.8	3.71	0.72
Apr.11-Apr.21	986.8	3.40	0.68	1272.2	2.84	0.43
Apr.22-May 1*	1185.8	2.80	0.55	1428.0	2.64	0.50
Average		3.26	0.65		3.06	0.55

*Relatively warm quarters may have reduced roughage intake.

The data in Table VI indicates that Lot II offspring consumed 6.5 percent less total digestible nutrients and 18.2 percent less digestible crude protein than did those in Lot I. This has un-

doubtedly affected the growth response adversely over the feeding period and thereby reduced the total gains for Lot II, particularly during the period between April 11th and 21st.

The quantity of total digestible nutrients and digestible crude protein ingested decreased per unit of body weight to the 0.73 power as the feeding trial progressed. While whole milk powder was fed to both groups, the offspring on non-leguminous rations ingested more total digestible nutrients and as much digestible crude protein as did those on the leguminous rations. However, with the removal of this ingredient from both rations about the middle of the second period (April 11th to 22nd), consumption of roughage did not increase correspondingly and a reduced nutrient intake was recorded. This was particularly true in Lot II as evident in Table VI.

Since the digestibility co-efficient of both roughages are practically the same, i.e. 53.4 and 53.9 percent respectively, the lower consumption of roughage by the non-leguminous fed group offspring must have been due to difference in palatability of the hays or a lowered appetite. The lack of significant difference in the total digestible nutrient intake during the last period (April 22nd to May 1st) suggests the cause of previous sub-normal consumption to be one of a temporary nature, probably adjustment to the less palatable ration.

Blood haemoglobin. Blood samples were taken when the trial had been completed. The blood of the legume-fed offspring showed an average haemoglobin content of 11.53 grams as compared with 9.78

grams for the offspring fed oat - prairie hay roughage, a difference of approximately 18 percent.

Despite the limited data on blood haemoglobin in the two lots, the readings taken are significant. In Lot II even the individuals which made good gains and which outwardly appeared as thrifty as animals in Lot I, showed the lower levels of haemoglobin recorded the offspring in Lot I. The difference of 18 percent in favor of the leguminous group definitely indicates the accumulative effect of a lack of some haematopoietic factor in the oat - prairie hay ration.

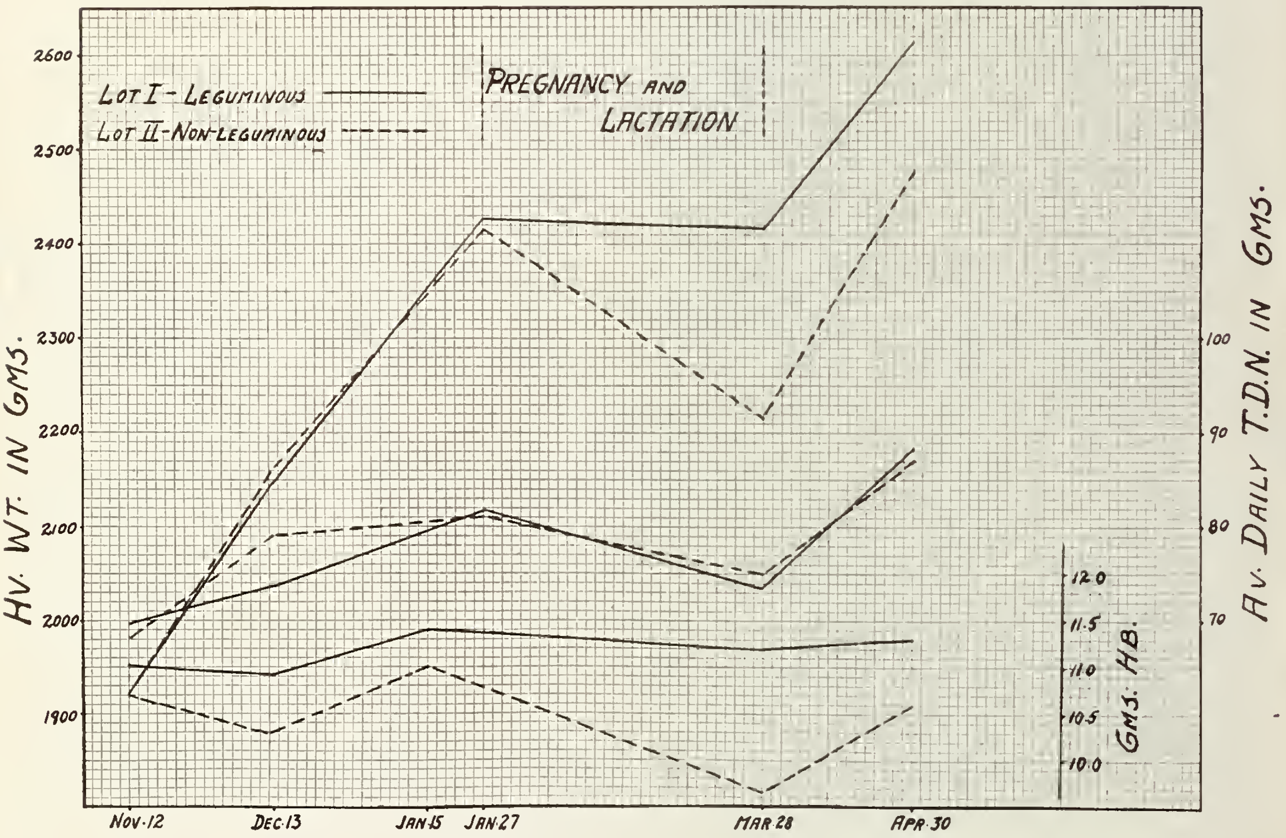
Discussion of Results.

Anorexia after prolonged dry feeding was the outstanding symptom observed in the sheep trials. Closely associated with this was the rapid decline in body weight which was particularly noticeable after roughage consumption fell to sub-maintenance levels. These symptoms were not noted in the experiment with rabbits. Table I presented data which indicate that in the case of the does the consumption of non-leguminous roughage was maintained throughout. This is also true for the offspring fed oat hay - prairie hay mixture.

While the non-pregnant weights of does in both lots were affected by lactation and the number of young suckled, there was no permanent and progressive decline. The rapid recovery in weight by does in Lot II would indicate that the weight loss was due to lactation rather than the effect of any specific deficiency.

GRAPH I

Average Weight, T.D.N. Intake and Blood Haemoglobin of Does on Leguminous and Non-Leguminous Rations
(on page 45)



Data relating to average body weight, feed consumption and blood haemoglobin of the does have been plotted in Graph I. The closeness with which the average blood haemoglobin fluctuation follows the trend of the average weight curve denotes a close correlation. It is apparent that the variation in body weight is influenced more directly by the strains to which the does were subjected than by the total digestible nutrient intake when the respective curves are studied. The nutrient intake was appreciably lowered when the quantity of oat groats fed was reduced. This is evident for the period ending March 28th.

Undoubtedly the average haemoglobin values in Lot I on the leguminous ration represent more nearly what may be regarded as normal values. The fact that the average blood haemoglobin in Lot II on non-leguminous diet was consistently lower would indicate that the oat - prairie hay mixture did not possess the same amount of certain substances conducive to haemoglobin formation, otherwise the levels should have been more nearly the same during the course of the first 65 days on experiment.

The observations made upon the effect of the rations upon reproduction are limited and variable and therefore are of no great significance insofar as the general problem is concerned.

Summary.

A comparison of the effect of two rations, leguminous and non-leguminous, was made with breeding does and their offspring.

1. Anorexia was not observed at any time on either ration as

shown by the uniformity of roughage intake in both parent and progeny trials.

2. Body weights of the rabbits were not influenced by the quality of the two rations but were affected by gestation, lactation and plane of nutrition.

3. The average blood haemoglobin readings, while slightly higher in the does fed alfalfa, were significantly higher in the corresponding offspring group.

4. The leguminous ration did not promote better reproductive performance than did the non-leguminous ration, though data in this regard are limited.

5. No significant difference in growth of the progeny groups on these two rations could be recorded.

Conclusions.

1. It is to be concluded from this study that female rabbits which are fed a deficient non-leguminous ration manifest only in part the characteristic syndrome of breeding ewes on a similar ration. The only similarity lies in lowered blood haemoglobin, whereas anorexia and subsequent weight loss are not typical symptoms of malnutrition as is the case in the larger species. No significant difference was noted in the reproduction of does due to insufficiency and variability of the data.

2. Alfalfa hay is a more satisfactory roughage than an oat-prairie hay mixture for breeding does in that it is conducive to the maintenance of higher blood haemoglobin.

3. A study of the response of weanling rabbits reveals that

the only difference in the accumulative effect of the leguminous and non-leguminous rations lies in the haematopoietic superiority of the leguminous ration.

GENERAL CONCLUSIONS

1. From the results of these studies it may be concluded that the characteristic syndrome of breeding ewes fed a non-leguminous ration does not develop in full in female rats and rabbits on similar rations.

2. Anorexia, the typical symptom in the ewes, is not shown by rabbits nor by rats when fed the non-leguminous rations used in these trials.

3. Rats exhibit a sub-normal reproductive ability as do sheep when fed a non-leguminous ration. Rabbits did not show the same reaction but this may have been due to factors other than differences in the rations.

4. The non-leguminous ration lacks a substance or substances that are necessary for the maintenance of higher haemoglobin levels in the blood of rabbits. In sheep fed a similar ration, lower haemoglobin levels develop subsequent to anorexia. No haemoglobin determinations were made with rats.

5. Rats and rabbits as individual species are only partially suitable for use in the continuation of the study of a non-leguminous ration which proved deficient for breeding ewes. This is due to their failure to develop symptoms comparable with those of sheep.

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